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A Dynamic Zn Site in *Helicobacter pylori* HypA – A Potential Mechanism for Metal Specific Protein Activity

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In order to import, transport, export, and maintain homeostatic levels of intracellular transition metal ions, organisms have evolved trafficking proteins that often exhibit a biological response to the binding of a particular metal. How proteins achieve this specificity is largely unknown. HypA is an accessory protein and a putative metallochaperone that is critical for supplying nickel to the active site of NiFe hydrogenases. In addition to binding Ni(II), HypA also contains a zinc site that has been suggested to play a structural role. X-ray absorption spectroscopy (XAS) was used to show that the zinc site changes structure from a S_3(O/N)-donor ligand environment to an S_4-donor ligand environment upon binding nickel. This provides a potential mechanism for discriminating Ni(II) from other divalent metal ions.

The use of reactive and potentially toxic transition metals in enzyme active sites depends on proteins that can acquire metals from the environment (*e.g.,* permeases), transport them inside cells and incorporate them into apoenzymes (*e.g.,* metallochaperones), and control their intracellular concentration (*e.g.* transcriptional regulators), often with great specificity for the cognate metal. How metal-specific responses are achieved is largely unknown, but appears to involve allosteric mechanisms that differ in the details of how the identity of the metal bound is communicated to the protein structure. HypA is one of three proteins (HypA, HypB, and SlyD) that have been identified in assisting in the incorporation of Ni(II) into NiFe hydrogenase in *Escherichia coli* (*Ec*). The proper functioning of HypA has also been implicated in the insertion of Ni(II) into urease in *Helicobacter pylori* (*Hp*). Our recent work at the NSLS focused on understanding the structural environments of both the Ni(II) ion and a structural Zn(II) ion present in *Hp*HypA with the goal of understanding how this protein discriminates Ni(II) from other divalent metal ions. Our study of the Zn(II) site has shown that changes in the ligand environment of this metal site that accompany the binding of Ni(II) may indicate to the protein that the correct metal [Ni(II)] has been bound.

Authors (from left, back) Jeff Martin, Robert Herbst, David Kennedy, Crisjoe Joseph, and Jonathan Leung; (front) Cecilia Doddi, Sharon Leitch, Michael Maroney, Khadine Higgins, Kelly Ryan, and Kerrie O'Brien

The amino acid sequence of *Hp*HypA (**Figure 1**) contains two conserved CXXC motifs that have been shown to be involved in binding the Zn(II) ion in *Ec*HypA. Our work is the first to show that in the absence of Ni, only three of these cysteines are coordinated to the Zn. The fourth ligand, identified only as a nitrogen- or oxygen-donor, is displaced upon the coordination of Ni(II) by the fourth cysteine residue. The Fourier-transformed EXAFS spectra (**Figure 2**) for the Zn(II) site clearly show this change in ligand environment upon Ni(II) coordination. The zinc site in the protein with no other metal bound is best described as involving an S_3(N/O) ligand donor-atom environment. Upon binding Ni(II), the zinc site structure changes

to one that is best described as an S_4 ligand donor-atom environment. These changes may represent either changes in the protein structure that constrain the zinc site structure, or a competition between the Zn(II) and Ni(II) ions for the N/O donor involved. Either case could lead to a mechanism for specific recognition of Ni(II) binding in HypA.

Analysis of XANES and EXAFS data show that the Ni(II) site contains six N/O-donor ligands that include 1-2 histidine imidazole groups. The coordination environment of Ni(II) in *Hp*HypA is very similar to the Ni(II) site in UreE, the only other characterized Ni-binding domain in a metallochaperone. The Ni(II) site in UreE is also six-coordinate and composed of N/O-donor atoms including two histidines. This binding motif may represent a general structure that nature has evolved for metallochaperones involved in the incorporation of Ni(II) ions in ureases, hydrogenases, and potentially other Ni metalloenzymes for which chaperones have yet to be identified.

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Hp  MHEYSVVSSLIALCEEHAKKNQAHKIERVVVGIGERSAMDKSLFVSAFETFREESLVCKDAILDIVDEKVELE
Ec  MHEITLCQRALELIEQQAAKHGAKRVTGVWLKIGAFSCVETSSLAFCFDLVCRGS-VAEGCKLHLEEQEAECW

    CKDCSHVFKPNALDYGVCEKCHSKNVIITQGNEMRLLSLEMLAE
    CETCQQYVTLLTQRVRRCPQCHGDMLQIVADDGLQIRRIEIDQE
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Figure 1. *Sequence alignment of E. coli and H. pylori HypA (potential metal ligands including two conserved CXXC domains are highlighted).*

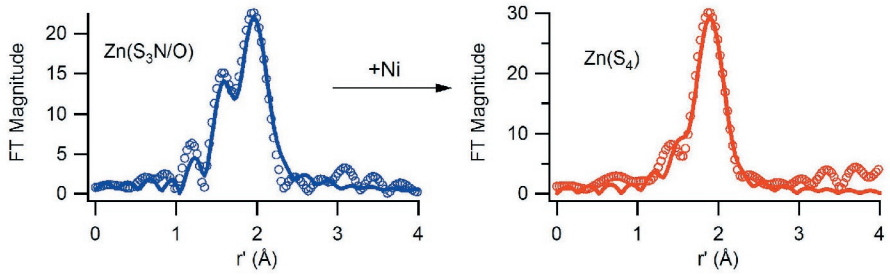

Figure 2. *The Fourier-transformed EXAFS spectra of the zinc site in the nickel-free (left) and nickel-bound (right) HypA proteins clearly showing the change in coordination environment at the zinc center.*